

Wound age and infection of peach bark by *Cytospora leucostoma*

A. R. BIGGS

Agriculture Canada, Research Station, Vineland Station, Ont., Canada LOR 2E0

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Peach bark wounds of varying ages were inoculated with mycelium of *Cytospora leucostoma* (Pers.) Fr., and colonization frequency and extent were determined. Noninoculated wounds of similar ages and in close proximity to inoculated wounds were also sampled and examined histologically for morphological and histochemical changes associated with nonspecific plant defense reactions, including lignification and formation of lignosuberized tissue and new periderm. Results demonstrated that lignified and lignosuberized tissues significantly decreased the rate of fungal colonization, whereas new periderm with at least three cells thickness of new phellem completely inhibited fungal colonization. The critical period regarding effective periderm formation was between 10 and 14 days postwounding.

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Des blessures d'âges varié effectuées sur l'écorce de pêchers ont été inoculées avec le mycélium de *Cytospora leucostoma* (Pers.) Fr.; par la suite, les fréquences ainsi que les étendues de colonisation ont été enregistrées. Des blessures non inoculées et d'âges similaires situées à proximité de blessures inoculées ont également été échantillonnées et soumises à l'examen histologique, afin d'observer les changements morphologiques et histologiques associés avec les réactions de défense non spécifiques de la plante, soit la lignification, la formation de tissus ligno-subérifiés ainsi que celle d'un nouveau périderme. Les résultats démontrent que les tissus lignifiés et ligno-subérifiés diminuent les taux de colonisation fongique, alors que le nouveau périderme comportant au moins trois couches de cellules de nouveau phellème inhibe complètement la colonisation fongique. La période critique pour la formation d'un périderme efficace se situe entre 10 et 14 jours après la blessure.

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Introduction

Peach canker, caused by *Cytospora cincta* (Pers.) Fr. (teleomorph = *Leucostoma cincta* (Pers.) Fr.) Hohn.) and *C. leucostoma* (Pers.) Fr. (teleomorph = *L. peroonii* (Nits.) Hohn.), is a major limiting factor in peach production in the northern portions of the region favourable for deciduous-tree fruit production in North America. Wounds created by leaf abscission, pruning, winter injury, and insect damage are the major avenues of entry for the peach canker fungi (19).

In many host-pathogen interactions, the boundary-setting process in wounds confers resistance to infection. Many researchers have demonstrated that wounds become increasingly less susceptible to infection with age (7, 8, 10, 13, 14). This type of resistance to infection is thought to be related to nonspecific plant

responses leading up to and including formation of primary lignosuberized tissues and secondary necrophylactic (wound) periderm (11, 12). The major structural components of these tissues are lignin and suberin (2, 3, 18).

There is no information available on the influence of wound age on susceptibility to infection of peach bark by *Cytospora* spp. With this understanding plus information on the influence of temperature on wound healing, the duration of susceptible periods that require fungicide prophylaxis could be predicted more accurately. If predictive equations could include the influence of cultivar (5) and water stress on wound responses even more accurate predictions could be made.

This paper describes the relationship between anatomical changes in bark and xylem

after wounding and the ability of *C leucostoma* mycelium to colonize wounds of varying age.

Materials and methods

Two-year-old nursery-grown peach trees (*Prunus persica* (L.) Batsch. cv. Loring) were dug in November 1983 and stored in a commercial nursery (Mori Nurseries, Ltd., Virgil, Ont., Canada) until the 1st week of January 1984. Twenty trees, transplanted into Vineland silt loam:peat:sand (20:9:6) in 30 cm diameter clay pots, were pruned to provide 50 cm of clear stem and 10 growing shoots per plant. Average length of the new shoots was approximately 9 cm at the beginning of the experiment. Greenhouse temperature was maintained in the range 22 to 30°C.

On March 1, 1984, all trees were sponged clean with distilled water to remove extraneous soil on the main stem. Each plant stem was measured and marked off to provide seven 7-cm segments. Each segment on each tree was randomly designated to receive one of seven wound-age treatments: inoculated at 0, 3, 7, 10, 14, or 24 days postwounding and a noninoculated wounded control. After wiping the bark with 70% ethanol, a sharpened 4 mm diameter cork borer was used to remove a portion of bark down to the xylem. Care was taken not to physically injure underlying xylem tissues while wounding. Wounds on each segment were done in pairs, each directly on the opposite side of the stem from the other.

Inoculations were made on 24 March when all 10 replicate trees had received six pairs of wounds ranging from fresh to 24 days old. One wound of each pair was inoculated with a 4 mm diameter malt agar disk of *C. leucostoma* and covered with cellophane tape for 3 days.

The seventh wound pair (also made on 24 March) was used as a noninoculated control, with one of the wounds receiving a plain malt agar disk. Preparation of the inoculum was as described previously (1). Canker length was

recorded at 3 days postinoculation and at weekly intervals thereafter.

The second (noninoculated) wound of each pair was excised on 24 March with a sterile razor blade, halved transversely, and placed immediately into formalin : acetic acid : alcohol (9). The tissue was processed and embedded in paraffin as described previously (1). Transversely oriented rotary microtome sections 8µm thick were assessed quantitatively for lignin and suberin in the boundary zone and necrophylactic periderm by using a Leitz MPV compact microscope photometer (3). Lignin was determined using the phloroglucinol + HCl reaction (9) and was quantified by measuring percent

transmittance of tissues at 540 nm. After measurements of percent transmission, phloroglucinol-treated tissues were examined using ultraviolet excitation (Leitz HBO 100-W mercury lamp, fluorescence filter block A) to detect and measure suberin autofluorescence intensity (4). For both per-cent transmission and autofluorescence intensities, three measurements were taken from serial sections on each slide at each of the six wound-age treatments. The entire experiment was performed twice with the second study initiated 2 weeks after the beginning of the first.

Additional data were collected on the timing and extent of reestablishment of lignin and (or) suberin continuity via lignified and (or) lignosuberized tissue. The number of necrophylactic phellem cells across the transverse thickness of the new periderm was determined at its junction with the original periderm. Suberized xylem ray parenchyma cells were observed and counted to determine the timing and extent of their formation in tissues directly internal to the w

Canker length was measured along the longitudinal axis of the stem. The data presented include the 4-mm wound. Control and noncolonized wounds always showed approximately 1 mm of necrosis around the wound. Fungal colonization rates for the different wound ages were determined from

weekly canker length measurements, using simple linear regression. Analysis of covariance and the F-test were used to determine homogeneity of regression coefficients. When regression coefficients were found to be heterogeneous, paired t-tests were used to separate wound-age differences (15).

Results and discussion

Data for the influence of wound age on canker length, measured at four postinoculation times, are presented in Table 1. Canker frequency was either 0 or 100% except for one infection of a 14-day-old wound. When canker length was measured 3 days postinoculation, only fresh and 3-day-old wounds showed necrosis significantly greater than at noninoculated wounds. Colonization of bark tissues by *C. leucostoma* in 3-day-old wounds was significantly less than at 0-day-old (fresh) wounds, suggesting that wound response events preceding delimitation of infections by lignosuberized tissue and necrophylactic periderm may be involved in nonspecific resistance to *Cytospora* spp. When canker length was assessed at 7, 14, or 21 days postinoculation, only 14- and 24-day-old wounds were not significantly different from the noninoculated control. Resistance to rapid fungal colonization became apparent between day 10 and day 14 in the wound healing process.

Rate of fungal colonization over the 21-day observation period was significantly influenced by wound age ($F = 9.31$, $P = 0.01$). The colonization rate at 0, 3, 7, and 10-day-old wounds was greater than at 14- and 24-day-old wounds, although colonization rate of fresh wounds was not significantly different from that of 3-day-old wounds (Table 1). It appears that in 3-day-old wounds the extent of fungal colonization is diminished relative to fresh wounds; however, the subsequent canker expansion rate for 3-day-old wounds would not differ significantly from that at fresh wounds.

Anatomical and morphological changes associated with wound-age treatments are

summarized in Table 2. The essential features of nonspecific responses of tree bark to injury or infection include the formation of a lignosuberized layer from cells present at the time of wounding, followed by differentiation of new phellogen immediately internal to the lignosuberized layer and, finally, differentiation of necrophylactic periderm. Bark response by day 3 postwounding was characterized by the presence of only lignin in the developing lignosuberized layer; by day 7 the lignosuberized layer was well formed, however, its continuity with the original suberized phellem was not observed; by day 10 the new phellogen and one new phellem cell were present at the junction of the new and original periderms; and by days 14 and 21 necrophylactic phellem was approximately three and six cells thick, respectively. Xylem ray parenchyma suberization was first observed in 7-day-old wounds and was not uniformly distributed in xylem tissues. Wound events in xylem did not appear to be related with colonization of bark tissues by *C. leucostoma*.

These data suggest that early, nonspecific responses to wounding, i.e., lignification and formation of lignosuberized tissue, decrease the rate of colonization rather than prevent colonization. Well-developed necrophylactic periderm, at least three phellem cells thick, acts to prevent colonization. In sweet potato, Walter and Schadel (17) reported that wounds were considered "healed" when wound periderm had attained a thickness of three to seven cell layers. Middleton and Bostock (10) reported that almond bark wounds became resistant to *Ceratocystis fimbriata* Ellis and Halst. infection within 10 - 14 days postwounding. The present study supports these findings and provides some perspective on the role of lignified and lignosuberized tissues after wounding. Tissues that formed before the necrophylactic periderm appeared to be easily penetrated by *Cytospora* species (1, 20) and the importance of mycelial aggregations in colonization by these and other fungi (6,16) should be considered.

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TABLE 1. Canker length (millimetres + 6.0 mm) and linear regression coefficients for the relationship between canker length (Y) and time (days, X) after inoculation of peach bark wounds of varying age with mycelium of *Cytospora leucostoma*

Wound age (days)	Wounds infected (%)	Time postinoculation (days)				Regression coefficient
		3	7	14	21	
CK	0	6.0*	6.0	6.0	6.0	0.388a+
24	0	6.2	6.0	6.0	6.2	0.395a
14	10	6.6	9.2	12.2	15.4	0.832a
10	100	6.6	13.0	18.6	24.6	1.277b
7	100	9.2	18.4	26.6	27.4	1.589b
3	100	14.2	20.2	26.2	30.2	1.705bc
0	100	20.2	27.2	39.2	49.8	2.656c

NOTE: CK, noninoculated control.

*Data are means of measurements from 10 trees from one experiment.

+Analysis of covariance for testing homogeneity of regression coefficients was significant, $F = 9.31$ ($P = 0.01$). Letters denote significantly different regression coefficients using paired t-tests in all combinations.

TABLE 2. Summary of morphological and histochemical changes related to phellogen generation after wounding in peach bark

Wound age (days)	Lignified boundary*	Lignosuberized boundary*	Necrophyllactic peridermT	Suberized xylem ray parenchyma*	% transmission (lignin)Tt	Autofluorescence intensity (suberin)§
0	---	---	0	---	100	0.0
3	+	---	0	---	97.3	0.0
7	+	+	0	+	76.7	3.1
10	+	+	1.0	+	66.0	15.2
14	+	+	3.0	+	67.1	21.2
24	+	+	6.0	+	61.4	33.3

*Feature present (+) or absent (-) in the outer bark cortex in contact with the original periderm in 100% of plants examined. For suberized xylem ray parenchyma, signs indicate presence or absence in 100% of plants examined.

T Mean number cells counted in transverse section at the junction of the new and the original periderms ($n = 20$).

Tt Percent transmission at 546 rim measured over a circular area with 272 μ m diameter; each measured area contained about 100 cells. Values represent mean percent transmission ($n = 20$) of lignosuberized tissue or lignosuberized tissue plus necrophyllactic phellem, depending on wound age. Nonwounded bark percent transmission = 100.

§Autofluorescence intensity measured over a circular area with 272 μ m diameter; each measured area contained about 100 cells. Values represent mean autofluorescence intensity ($n = 20$) of lignosuberized tissue or lignosuberized tissue plus necrophyllactic phellem, depending on wound age. Nonwounded bark autofluorescence intensity = 0.